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Understanding Dynamic Molecular Responses Is Key to Designing Environmental Stress Experiments: A Review of Gene and Protein Expression in Cnidaria Under Stress

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ABSTRACT

Gene and protein expression analyses are powerful tools to investigate the responses of cnidarians to stress, providing information on both genetic and functional variation and capturing dynamic shifts in organismal physiology. As the use of high throughput sequencing to understand responses of cnidarians to stressors is still relatively new, standard experimental protocols have not yet been established, which limits the ability to compare studies. We (1) systematically reviewed the literature of cnidarian gene and protein expression studies related to environmental stressors to determine how the laboratory experiments were designed and (2) investigated the consistency in responses of genes commonly used as biomarkers within stress experiments conducted on the five most-studied cnidarian genera. Duration of exposure to the stressor, acclimation period and intensity of stress varied greatly among experiments, and most studies did not sample during acclimation and recovery. Before designing experiments that aim to characterise molecular responses to a specific environmental stress, research efforts need to focus on understanding the plasticity of whole transcriptome responses, as gene expression can vary under different stress intensities and durations of exposure. Additionally, only seven genes that were tested in at least two different genera showed a consistent response under heat stress (CuZn-SOD, c-type lectin, FGFR1, MMP, Zn-MP, NF-κB and SLC26). These genes have the potential to standardise evaluations of temperature stress across experiments on cnidarians, and we suggest exploring their use as general cnidarian biomarkers of temperature stress (cBATS).

1 | Introduction

1.1 | Phenotypic Plasticity and Tolerance to Environmental Stress

Environmental stressors are changes in the environment that reduce an organism's performance or fitness (Schulte 2014). There is major concern regarding how incremental changes in global scale environmental stressors, such as climate change, interact with acute local-scale stress events to affect survival and distributions of species. Species/populations can experience stress differently, and their responses can depend on the type of stressor and its intensity and duration (Beusen et al. 2022). Species that are widely distributed and/or inhabit extremely variable environments often are more tolerant to stress (Box 1; Niinemets and Valladares 2008). As the stress experienced by

BOX 1 | Definitions of Key Terminology.

Tolerance = ability to survive and grow under stress.

Sensitive species/population = with low tolerance.
Acclimation = ability of an organism to adjust its phenotype to new environmental conditions over the course of its lifetime.

Transcriptome/proteome = complete set of RNA transcripts/proteins produced by a cell, tissue, or organism at a certain developmental stage and/or under a specific physiological condition.

Transcriptomic plasticity = variation in gene expression due to differences in environmental conditions.
Transcriptomic resilience = ability to return to a baseline of gene expression after stress.

Biomarkers of stress = biochemical, cellular, or physiological variations that can be measured in tissue or body fluid samples, or at the level of whole organisms, to provide evidence of exposure and/or effects from one or more stressors.

an animal is a function of both the duration and intensity of the stressor, this threshold of tolerance would also depend on those features (Schulte 2014). Even species that tolerate high intensities of a stressor can still experience stress if a level of stress intensity is maintained for a long time.

Species can develop tolerance to novel environmental conditions through phenotypic plasticity and/or by genetic adaptation (Fox et al. 2019; Blewett et al. 2022). Phenotypic plasticity allows a single genotype to manifest different phenotypes in divergent environments, while adaptation enhances fitness in an environment through genetic changes across generations (Fox et al. 2019; Leeuwis and Gamperl 2022). A plastic response may be temporary and reversible within an animal's lifetime. For example, *Amphiglena mediterranea* (Leydig, 1851), a tolerant polychaete that inhabits low pH environments, can quickly acclimate (Box 1) to higher pH environments by adjusting its metabolic rates (Calosi et al. 2013). Organisms with greater plasticity in environmental stress responses can acclimate faster to new conditions and recover from stress more quickly (Pazzaglia et al. 2021).

1.2 | Studying Responses to Environmental Stress in Laboratory and Field Experiments

The responses of organisms to environmental stressors can be tested though ecological experiments in the field or laboratory. Manipulative field experiments tend to be more realistic because organisms experience natural biological interactions and fluctuations in all environmental factors that are not being manipulated. Nevertheless, developing appropriate experimental designs that accurately manipulate the stressor of interest and include adequate replication and sampling in the field can be challenging (Connell 1974; Raffaelli and Moller 1999; Filazzola and Cahill 2021). Although it is more difficult to simulate natural conditions in the laboratory (i.e., it is difficult to accurately reproduce environmental conditions, and knowledge of husbandry is required), laboratory-based experiments are more feasible and enable independent variables to be controlled more precisely (Diamond 1983). To be environmentally relevant, laboratory experiments need to mimic field conditions to the extent possible. For example, the intensity of stress, the rate at which it is introduced, and the duration for which it is applied should reflect actual events. This is important because simulating unrealistic scenarios could lead to inappropriate conclusions about the responses of species to environmental stressors and have limited power for predicting future scenarios (Hughes et al. 2017; Sucharitakul and Pitt 2022).

When organisms need to be handled before an experiment, it is common to acclimate the specimens to the experimental conditions to enable them to recover from the stress of being collected or manipulated. As species/populations recover from stress at different rates, acclimation can vary from one species/population to another (Vinagre et al. 2016). Failing to allow organisms to acclimate to experimental conditions could result in experiments commencing with the animals already stressed, compromising the results. For that reason, knowing how long an animal takes to acclimate and/or recover after stress is essential to understand how plastic the response to environmental stressors is.

As advantages and disadvantages exist for both field and laboratory experiments, the choice of which location to use to study how animals respond to environmental stress should depend on the research question and feasibility of the experiment, with the limitations of methodological designs clearly indicated. This is important because different, and even contrasting, results are sometimes obtained from laboratory-based and field-based experiments designed to test the same hypothesis (Calisi and Bentley 2009), so limitations and assumptions of each approach must be carefully considered to ensure results are interpreted appropriately.

1.3 | The Use of Gene and Protein Expression as Tools to Understand Responses to Environmental Stress

Changes in physiology, metabolism and behaviour are relatively straightforward to measure and are commonly used to assess stress. Those response variables usually manifest after changes at the molecular/cellular level, and, therefore, may not be suitable for detecting subtle or rapid responses to stress. Gene and protein expression analyses have emerged as highly informative tools to study those responses (DeBiasse and Kelly 2016), as they often precede and mediate changes in physiology or behaviour (e.g., Wellband and Heath 2017). The analysis of changes in global gene expression profiles under different conditions (the 'reaction norm', see Rivera et al. 2021) can be used in stress experiments to reveal transcriptome plasticity of responses to stress, and the organism's resilience to the stressor (Box 1). The expression of single genes can also be compared (e.g., qPCR-based analysis) under different environmental conditions to investigate the link between this gene and the stress response. Analysis of changes in global protein expression (quantitative proteomics) and single protein expression (e.g., western blots) can also be used

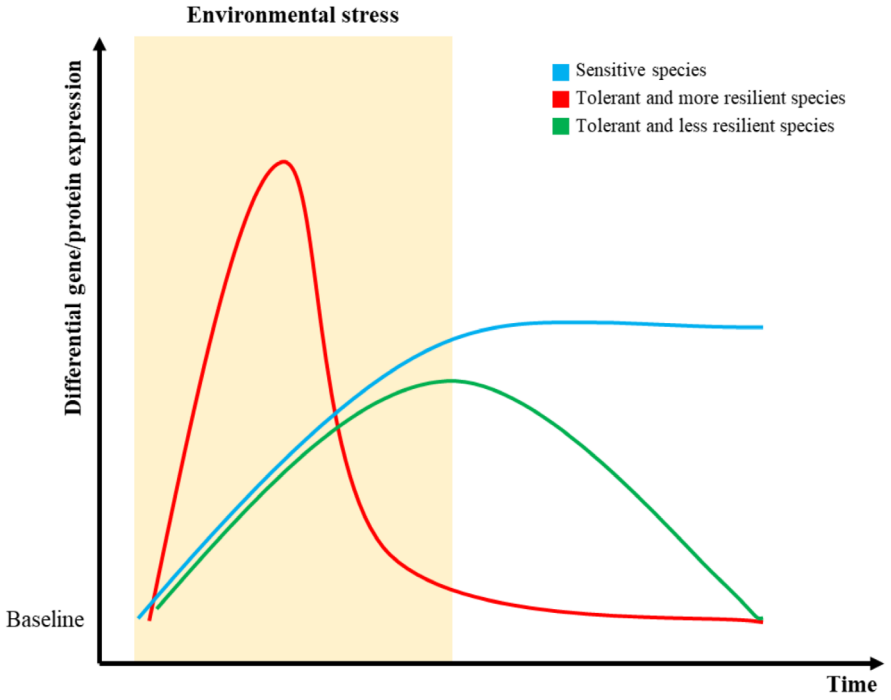

FIGURE 1 | Possible variation in differential gene expression of tolerant and sensitive species under stress, considering that all species have the same baseline of gene expression. Inspired by Rivera et al. (2021).

to assess if an organism is stressed, with the added benefit of capturing post-transcriptional processes that may alter protein, but not mRNA, levels. Although proteomic studies are still less common than transcriptomic studies due to their higher complexity (e.g., variations in protein abundance, hydrophobicity, stability, size and charge) and cost, recent advances have been made in mass spectrometric techniques and single-cell proteomics (Bennett et al. 2023), which will make these analyses more accessible moving forward.

Knowledge of the plasticity and resilience of an organism to specific stressors can be used, for example, to optimise the frequency of sampling throughout an experiment. This optimisation is especially important when utilising whole transcriptome expression as a response variable, due to the dynamic nature of global expression. As intensity and duration of stress can influence gene expression patterns (e.g., Dixon et al. 2020), designing stress experiments without knowing how plastic and resilient the species/population is to a specific stressor can be challenging. While transcriptome plasticity allows the organism to adjust expression profiles to tolerate the stress, resilience enables it to return to a baseline of expression (Figure 1). Even species that can tolerate an environmental stressor can vary in their rate of stress response (Rivera et al. 2021), with a fast (tolerant and extremely resilient species) or slow (tolerant but less resilient species) change in global differential gene expression.

Reduced transcriptome plasticity is not always associated with low tolerance to stress. For example, no transcriptomic change was detected in the reef-building coral *Acropora nana* (Studer, 1879) when exposed to heat, exhibiting a low plasticity but high tolerance to stress (dampening) (Bay and Palumbi 2015). Tolerance can also be achieved by sustaining a higher baseline of gene expression with reduced plasticity upon exposure to stress (frontloading), commonly associated with populations that live in highly variable environments (Barshis et al. 2013; Rivera et al. 2021).

1.4 | Genes and Proteins as Biomarkers of Environmental Stress

The use of gene and protein expression as biomarkers of stress (Box 1) has been increasing in management and conservation (Lam 2009; Parkinson et al. 2020). By comparing the transcriptome of different organisms/tissues before, during and after exposure to stressors, stress response genes can be identified for use as biomarkers in later experiments. Although proteomics methods are still underdeveloped in comparison with transcriptomics, protein-based biomarkers can also be optimised through western blotting analysis, focussed on specific proteins of interest (e.g., Downs et al. 2000). However, genes and proteins can be upregulated or downregulated depending on the duration and intensity of stress, and this regulation can also vary among species (e.g., Louis et al. 2017) and populations within the same species (e.g., Fangue et al. 2006). Biomarkers, therefore, require validation within these different contexts to assess if their expression profile is consistent and representative of the global transcriptional/proteomic profile of the taxa under a specific stressor (Figure 1). Establishing reliable and consistent biomarkers of stress that could be used across different taxa is still a goal of ecologists, but some genes are promising candidates. These include genes that encode for heat shock proteins (HSPs), oxidative stress response (such as superoxide dismutase, manganese superoxide dismutase, ferritin and catalase) and immune response (such as the transcription factor Nf-kβ) (Louis et al. 2017).

Heat shock proteins, superoxide dismutase (SOD), catalase (CAT) and ferritin have also been indicated as good protein biomarkers of stress, together with fluorescent proteins (FPs) and monooxygenase enzymes (such as NADPH cytochrome *c* reductase) (Solayan 2016). Measuring molecular responses from a greater diversity of species and stress conditions will help to determine whether these genes and proteins can be used as biomarkers.

1.5 | Responses of Cnidarians to Environmental Stress

In the context of marine ecology, reliable biomarkers of stress could be used to formulate management approaches to conserve coral reefs (Lesser 2011). Climate change is increasing the frequency, duration and intensity of extreme temperature events (Oliver et al. 2018), which are responsible for declines in coral cover and phase shifts in community structure of coral reefs (West and Salm 2003). As coral reefs have high conservation value, effort has been directed to understand how corals, the building blocks of coral reefs, respond to different environmental stressors. Nevertheless, corals belong to a diverse phylum (Cnidaria Hatschek, 1888) that also includes anemones, jellyfishes, hydras, siphonophores and myxozoan endoparasites. Studying different cnidarian taxa could help inform about diversity and evolution of stress responses and understand the molecular mechanisms behind those responses.

Laboratory experiments are commonly used to investigate the effects of environmental stressors on cnidarians (e.g., Edge et al. 2005; Goldstone 2008; Wright et al. 2019; Bass et al. 2021). Nevertheless, the designs of experiments that test stress responses of those animals vary greatly (e.g., McLachlan et al. 2020) and are sometimes poorly described in published papers. With the advent of new, more comprehensive and affordable molecular sequencing technologies, the use of gene expression as tools to investigate the responses of cnidarians to environmental stress is rapidly increasing (Maor-Landaw and Levy 2016; Rivera et al. 2021; Kenkel and Wright 2022). Considering that the use of these tools to assess cnidarians' responses is still relatively new, no adequate procedures have been established to help optimise experimental designs so that they are statistically rigorous and accommodate the dynamic nature of gene and protein expression.

To facilitate the design of rigorous experiments that evaluate responses to environmental stress in cnidarians, we systematically reviewed the literature of cnidarian gene and protein expression studies related to environmental stressors. Our main goals were to: (1) review the types of environmental stressors, clades and species that were studied; (2) examine how the laboratory experiments were designed, especially regarding the intensity and duration of stress exposure, sampling frequency and inclusion of acclimation and recovery periods; and (3) investigate the consistency with which genes that are commonly used as biomarkers are differentially expressed by comparing results of transcriptomics and single gene expression-based heat stress experiments on the five most studied Cnidarian genera.

2 | Methods

The literature was systematically reviewed using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 method (Page et al. 2021). The Web of Science was searched for articles published prior to December 31st, 2022. The following terms were searched within the title, keywords and/or abstract of publications: ('Cnidaria' OR 'Anthozoa' OR 'coral' OR 'anemone' OR 'Hydrozoa*' OR 'Hydra' OR 'Scyphozoa*' OR 'medusa*' OR 'jellyfish' OR Myxozoa* OR Staurozoa*) AND ('stress*' OR 'response') AND ('environment*' OR 'temperature' OR 'climate' OR 'acidification' OR 'heat' OR 'salinity' OR 'hypoxia' OR 'pH') AND ('transcript*' OR 'protein*' OR 'gene' AND 'expression'). This search returned 745 publications, which were manually reviewed and excluded if they: (1) did not conduct a gene or protein expression study (including whole transcriptomic and proteomic analysis, differential gene or protein expression and single-gene or single-protein expression studies) generating original data; (2) did not have a hypothesis associated with environmental stress (articles that focussed on disease and symbiotic interactions were excluded if the analysis was not associated with an environmental stressor); (3) did not include cnidarians; (4) were reviews or methodological briefs.

The remaining 307 publications (Appendix S1) were assessed for: (1) species and class or subclass of organisms; (2) type of study (laboratory-based experiment, field-based experiment or ex situ observation, without experimental component); (3) molecular approach (gene expression or protein expression); (4) type of stressor(s); (5) duration of exposure to stressor; (6) period of acclimation prior to exposure to experimental conditions; (7) period of recovery from stress exposure; (8) intensity of stress; (9) rate at which the stressor was increased; (10) justification provided by the authors for stress intensity (only for laboratory-based experiments); and (11) the purpose of the study. Taxa were classified as per the World Register of Marine Species (WoRMS). The environmental stressors were classified as: temperature variation; chemical pollutants (including pesticides, drugs, metals, nanoparticles and eutrophication); microplastics; hypoxia; acidification/pH variation; sediment deposition; salinity variation; starvation; light variation/UV exposure; air exposure; and undefined stressors (field studies that could not isolate specific stressors). Intensity of stress in temperature variation experiments was measured as the difference between the temperatures used in the control and minimum temperature treatment. The purposes of the studies were categorised as: 'acclimation, resilience and recovery'; 'characterization and/or quantification of DEG/DEP'; 'comparative response of species/populations'; and 'other' (Appendix S2).

The duration and intensity of stress experiments and duration of acclimation were analysed using the computing software R version 4 (2022). Those variables were compared between studies according to the purpose of the study, type of stressor, five most studied genera and justification for stress intensity provided by the authors. Variables compared among studies were treated as independent. Levene's test was used to assess the homogeneity of variances. When variances were not significantly different ($p < 0.05$) one-way ANOVA tests were used. When variances

were significantly different, non-parametric Kruskal-Wallis tests were used.

Forty-two articles that investigated the effects of heat stress in the five most studied genera were analysed to determine whether any genes are consistently identified as differentially expressed across studies. These articles were surveyed for differentially expressed genes (DEGs) identified by the authors during stress exposure in comparison to control treatments. Articles that focussed on larvae and did not have temperature as a separate stressor (when two or more stressors were assessed simultaneously) were excluded from this analysis. All genes identified by the authors were recorded (Appendix S3) and categorised as 'upregulated', 'downregulated' or 'no significant change', according to the results reported by the authors after differential expression analysis. Genes that were identified in at least two experiments were selected, and their differential expression was compared between studies to assess their suitability as possible biomarkers of heat stress for cnidarians (Table 1). Duration and intensity of stress were also compared across studies and categorised as 'extra-short' (≤1 day), 'short' (2–7 days), 'medium' (8–14 days), 'long' (15–30 days), or 'extended' (> 30 days) duration and 'low' (2°C–3°C), 'moderate' (4°C–5°C), 'high' (6°C–8°C), or 'extreme' (9°C–10°C) intensity. To determine whether any genes were consistently differentially expressed (i.e., to identify potential stress biomarkers), values were attributed to each gene under different experimental conditions according to their response: upregulated, 1 point, downregulated, −1 point, no significant change, 0 points. An 'index' of tendency of genetic response was established by calculating the average of those values under different conditions of heat stress across studies (Table 2). A positive index value indicated a tendency for upregulation, a negative index value indicated a tendency for downregulation, and a 0 value indicated a tendency for no differential expression of the gene under the experimental conditions analysed.

3 | Results

The number of studies that use gene and protein expression analysis to assess the response of cnidarians to environmental stress has increased 10-fold since 2002 (Figure 2A), with gene-based studies increasing three times more than protein-based studies. Most studies have focused on anthozoan species (88%), which represent about 67% of cnidarian species, while 11% of studies focused on hydrozoans (which represent 25.5% of cnidarian species) and 1% have focused on scyphozoans (1.6% of cnidarian species) (Figure 2B). Most (57%) Anthozoa studies focused on four genera: *Acropora* (31% of studies), *Seriatopora* Lamarck, 1816 (10% of studies), *Montastraea* Blainville, 1830 (8% of studies) and *Porites* Link, 1807 (8% of studies). Ninety percent of the publications on Hydrozoa were on the freshwater genus *Hydra* Linnaeus, 1758. Four of the five studies on scyphozoans were on *Aurelia* spp. Lamarck, 1816, and one was on *Stomolophus meleagris* Agassiz, 1860. No gene or protein expression studies were found for the cnidarian classes Cubozoa, Myxozoa and Staurozoa.

Temperature was the most studied stressor (46.6% of the 307 publications assessed) (Figure 3A), followed by 'undefined stressors' (field studies that could not isolate specific stressors; 14.9%), 'chemical exposure' (11.6%) and 'acidification' (11%). 77% of experimental studies analysed the responses of cnidarians to a single stressor and among the publications that considered more than one stressor, the most common combination (32%) was temperature and pH (Figure 3B).

Duration of exposure to the stressor and acclimation periods varied greatly between lab-based experiments and there was no difference between protein-based and gene-based studies (Figure 4A–D). The acclimation period before initiating the experiment was not significantly different between the five most studied genera (*Acropora*, *Montastraea*, *Pocillopora*, *Porites* and *Stylophora*), varying from 2 days to more than a year (ANOVA: $F = 0.582$, $p = 0.676$, Figure 4A). Duration of acclimation did not vary significantly according to the purpose of study (ANOVA: $F = 1.277$, $p = 0.287$, Figure 4B). Duration of exposure to the stressor was significantly different between different purposes of research (One-way ANOVA: $F = 5.362$, $p = 0.005$, Figure 4C), but not between different types of stressors (Kruskal–Wallis test: $p = 1.751$, Figure 4D). Experiments that investigated 'acclimation, resilience and recovery' exposed organisms to stressors for an average of 41 days, while experiments that focused on 'characterization/quantification of DEG/DEP' exposed the animals for an average 11 days, and experiments that focused on 'comparison of species/populations response' for an average of 19 days (Figure 4C). Only 12% of the experiments sampled after a recovery period (excluding articles that investigated 'starvation' and 'undefined stressors').

Thirty percent of all lab-based experiments did not introduce the stressor gradually (i.e., animals were transferred immediately to the intended stress intensity without exposure to any intermediate conditions) and the rate of increase was not stated in 20% of the studies. Most studies (75%), that either introduced the stressor slowly or did not state the rate, focused on 'characterizing and quantifying DEG and DEP', and 43% did not justify the stress intensity.

Since temperature variation was studied four times more often than other stressors, we only compared the intensity of stress within temperature studies (Figure 5). Thirty-one percent of temperature stress studies used more than one intensity of stress. Mean stress intensity was lower in experiments that used the 'IPCC report' to justify the intensity used (One-way ANOVA: $F = 4.896$, $p = 0.00852$, Figure 5A), but did not change between different purposes of study (One-way ANOVA: $F = 0.4$, $p = 0.753$, Figure 5B).

As temperature was the most studied stressor, we compared DEGs identified by the authors in heat stress experiments on the five most studied genera: *Acropora* Oken, 1815, *Montastraea* Blainville, 1830, *Pocillopora* Lamarck, 1816, *Porites* Link, 1807, *Stylophora* Schweigger, 1820. Forty-two of the 56 genes identified in at least two different studies responded inconsistently to heat stress (Appendix S3). Fourteen genes showed a consistent response to heat stress (Table 1). Twenty-eight genes were studied in at least four experiments, and only one of those, NF-κB, was consistently regulated across studies (Table 2).

TABLE 1 | The 14 genes identified with consistent differential expression in at least two experimental conditions that analysed heat stress on corals.

Species	Publication	Duration (days)	Intensity (°C)	AP-1	Chorion peroxidase	C-type lectin	CuZnSOD	Cytochrome c oxidase	Cytochrome b	FGFR1a	HSP40	MMP	Zn-MP	NADH	NF-κB	Tyrosil-tRNA	SLC26
Acropora aspera	Rosic et al. (2014)	1	6														
	Rosic et al. (2014)	3	6														
Acropora cervicornis	Parkinson et al. (2018)	< 1	9														
Acropora hyacinthus	Fu et al. (2022)	28	3														
	Traylor-Knowles et al. (2017)	< 1	6														
Acropora millepora	Bellantuono et al. (2012)	8	10														
	Császár et al. (2009)	9	5														
	Császár et al. (2010)	15	5														
	Souter et al. (2011)	9	5														
Acropora palmata	DeSalvo et al. (2010)	2	3														
Montastraea cavernosa	Lesser et al. (2020)	12	3														
Montastraea faveolata	DeSalvo et al. (2008)	10	3														
Pocillopora acuta	Gösser et al. (2021)	< 1	9														
	Poquita-Du et al. (2019)	4	2														
Pocillopora damicornis	Vidal-Dupiol et al. (2009)	15	4														
Porites astreoides	Kenkel et al. (2013)	42	4														
Stylophora pistillata	Bernardet et al. (2019)	7	7														

Note: Blue = downregulated. Red = upregulated. Empty spaces correspond to the absence of data.
Abbreviations: AP-1, activator protein 1; CuZnSOD, copper-zinc superoxide dismutase; FGFR1a, fibroblast growth factor receptor 1a; HSP40, heat shock protein 40; MMP, matrix metalloproteinase; NADH, nicotinamide adenine dinucleotide; NF-κB, nuclear factor kappa B; SLC26, solute carrier family 26; Zn-MP, metalloprotease Zn^{2+}.

TABLE 2 | Tendency for differential regulation of genes analysed in at least four experiments according to the duration of the experiment, the intensity of heat stress and the genus studied.

	Duration of experiment					Intensity of heat stress				Genus					Total number of experiments		
Genes	Extra-short (≤1 day)	Short (2–7 days)	Medium (8–14 days)	Long (15–30 days)	Extended (>30 days)	Low (2°C–3°C)	Moderate (4°C–5°C)	High (6°C–8°C)	Extreme (9°C–10°C)	*Acropora*	*Montastraea*	*Pocillopora*	*Porites*	*Stylophora*	Upregulated	Downregulated	No change
Actin	−0.5	−1.0	−1.0		0.0	−0.7	−0.5	−1.0				−1.0	−0.6		0	4	2
APAF-1	1.0	0.5							0.8					0.8	3	0	1
Bax	1.0	0.0	1.0			1.0			0.6	1.0	1.0			0.5	4	0	2
Bcl-2	0.8	0.6	0.0	0.0		0.0		0.4	0.7	1.0	0.2	1.0		0.6	8	0	8
BI-1	1.0	0.0							0.5					0.5	2	0	2
C3	0.0	−1.0	1.0		0.0	0.0	0.3	−1.0		1.0			−0.3		1	1	3
CA	−1.0	0.0			−1.0		−0.3	−1.0		1.0	−1.0	0.0	−1.0	−1.0	1	4	1
Caspase	1.0	0.4	0.4	1.0		1.0	0.0	0.7	0.7	0.8		1.0	0.3	0.5	11	0	8
Catalase	0.5	−0.5	−0.3	0.5		0.5	0.0	−0.1		0.5	−0.3				2	2	6
CatL	0.0	0.0	−0.5	0.0			0.0	−0.2			−0.2		0.0		0	1	5
DnaJ C3	1.0		0.5				0.0	1.0	0.6	0.3		1.0	0.3	0.8	6	0	5
Enolase			0.3				0.3	0.3	0.3	0.3			0.3	0.3	3	0	7
Ferritin			0.7	1.0			0.7		1.0	0.8					3	0	1
GAPDH	0.0	0.0	1.0			0.0	0.3	1.0		0.7		0.0	0.0		2	0	4
GFP-like	−0.5	−0.3	0.0		0.0	0.0	−0.3	−0.3	−1.0	−1.0	0.0	−1.0	0.3		1	4	4
HSP16	1.0	0.7	1.0		0.0	0.8	0.5	1.0	1.0	1.0		0.5	0.8		6	0	2
HSP60	0.5	1.0			0.0	0.0	0.5	1.0					0.5		2	0	2
HSP70	0.7	0.4	0.5	1.0	0.0	0.0	0.4	0.9	0.6	0.7	0.0	0.6	0.7	0.4	14	0	12
HSP90	0.5	0.4	0.2	0.0	−1.0	0.5	0.3	0.2		0.7	−0.3	1.0	0.3		9	4	5
MnSoD	1.0	0.0	0.7	1.0		0.0	1.0	0.5		0.8	0.0				4	0	0
NF-κB	1.0	1.0	1.0			1.0	1.0		1.0	1.0		1.0			3	0	6
Prx6			0.3				0.7	0.0	0.3	0.3			0.3	0.3	2	0	8
RAD51			0.2				0.3	0.0	0.3	0.3			0.0	0.3	1	1	3
RpS7	0.0	0.0	0.0	0.0				0.0			0.0				1	2	2
RpL9	−1.0	0.0	0.0	0.0				−0.2			−0.2				1	0	3
Survivin	0.0	0.5							0.3					0.3	4	0	2
TRAF	0.4	1.0	−1.0			1.0	0.0	1.0	0.0	0.2		1.0	0.0		6	2	1
TXN	0.3	−1.0	0.5				0.2	0.3	0.5	0.3	0.0		0.3	0.5	6	1	8

Note: Values represent the mean DE tendency for each gene (see Section 2). Empty spaces correspond to the absence of data. Blue = tendency for downregulation. Red = tendency for upregulation. Publications that performed more than one experiment are represented independently for each experimental condition.

Abbreviations: APAF-1, apoptotic protease activating factor 1; Bax, Bcl-2 associated X protein; Bcl-2, B-cell lymphoma 2 protein; BI-1, Bax inhibitor-1; C3, complement component 3; CA, carbonic anhydrase; CatL, cathepsin L; DnaJ C3, heat shock protein 40 member C3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFP-like, green fluorescent chromoprotein; HSP, heat shock protein; MnSoD, manganese superoxide dismutase; NF-κB, nuclear factor kappa B; Prx6, peroxiredoxin-6; RAD51, DNA repair protein RAD51; RpL9, ribosomal protein subunit L9; RpS7, ribosomal protein subunit S7; TRAF, tumour necrosis receptor-associated factor; TXN, thioredoxin.

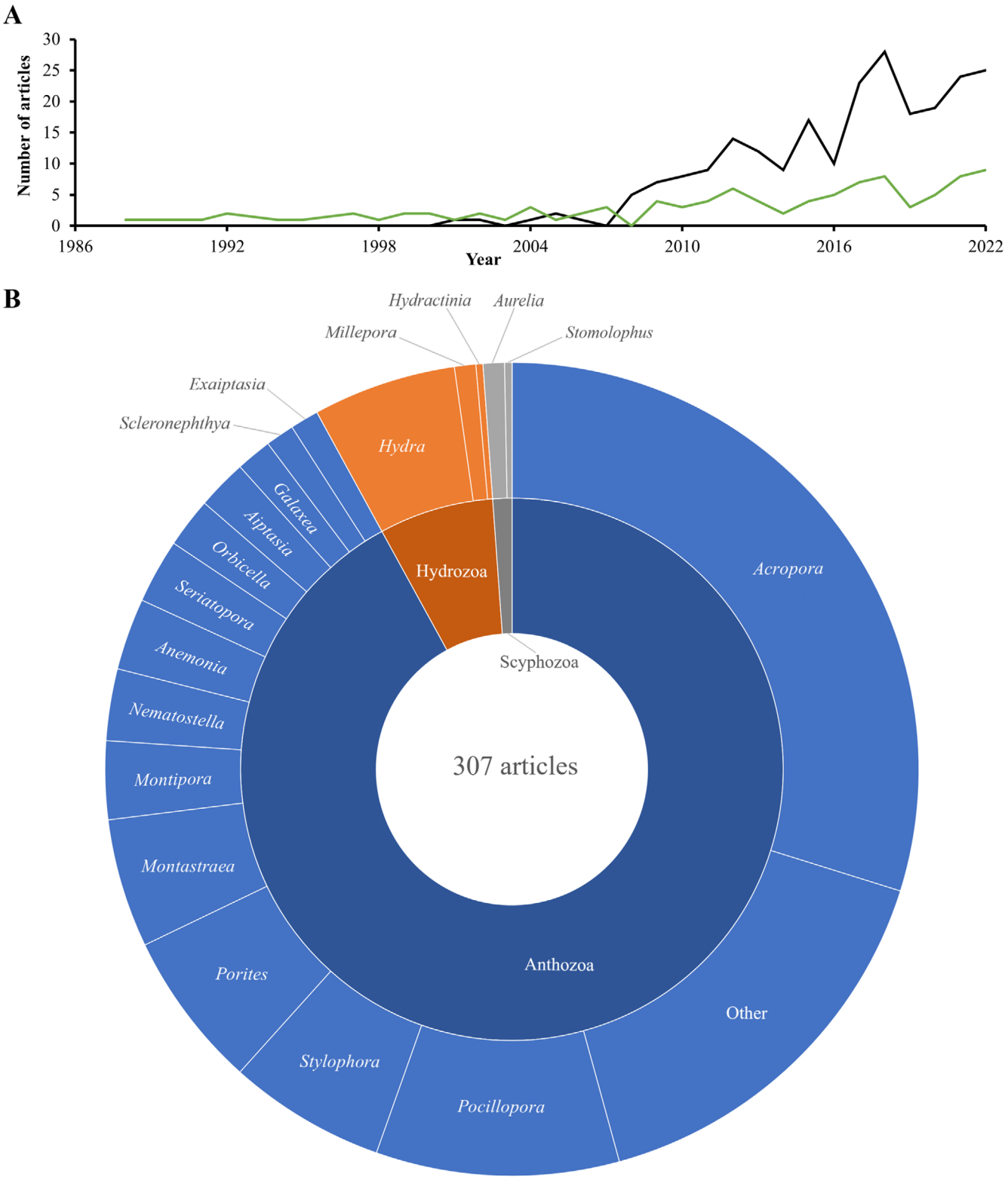

FIGURE 2 | (A) Number of publications per year on cnidarian gene (black line) and protein (green line) expression related to environmental stressors; articles that assessed both gene and protein expression are represented independently for each analysis. (B) Taxonomic classification of studied organisms, with the inner circle representing Class and the outer circle Genus. 'Other' represents Anthozoan genera with < 1% of representation. Publications that consider more than one species are represented independently for each taxon.

4 | Discussion

The capacity of species/populations to display transcriptomic (and, consequently, phenotypic) plasticity will determine their success under future anthropogenic environmental change (Fox et al. 2019). Transcriptomic plasticity can be investigated in laboratory experiments through two main aspects of the experimental design: (1) the dynamics of response to stress;

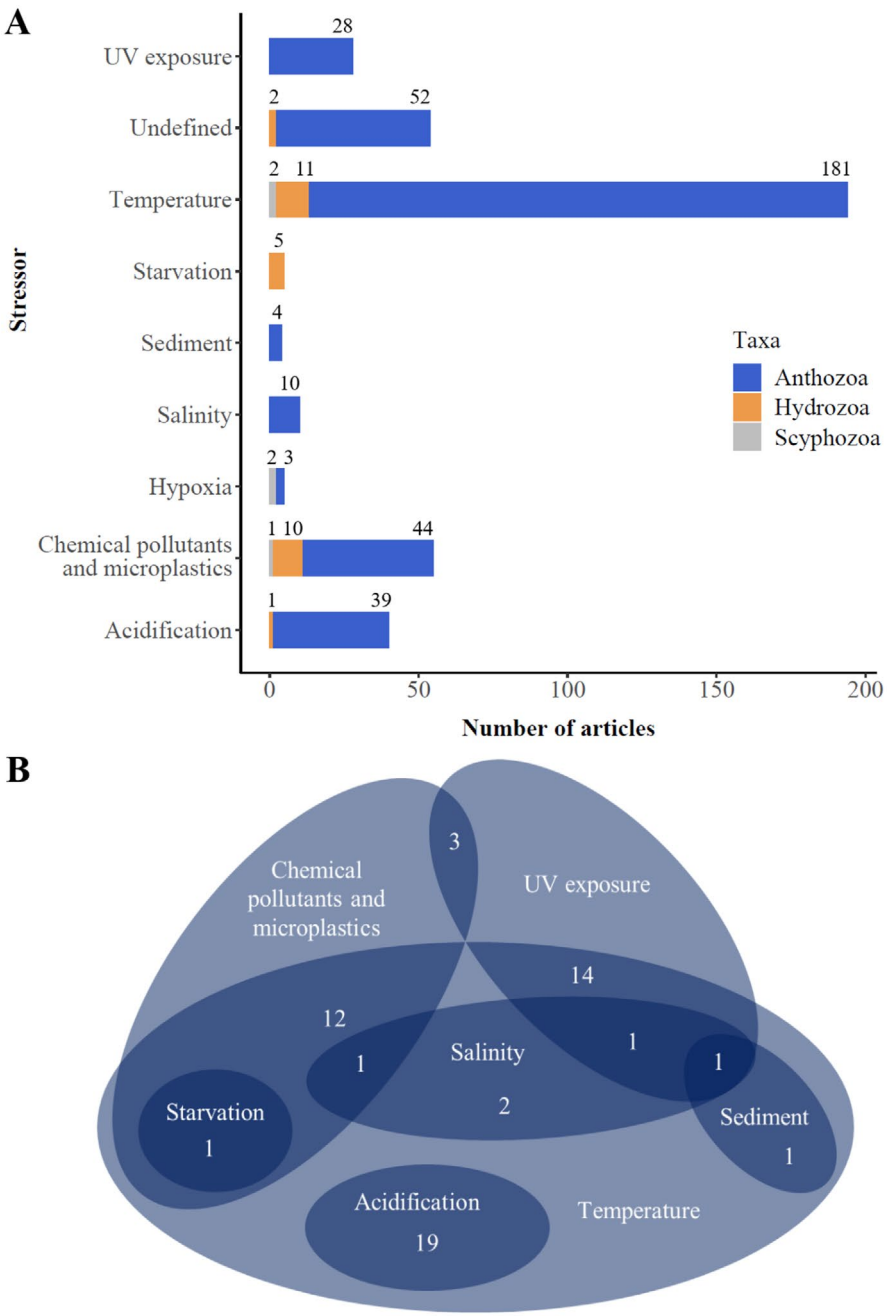

FIGURE 3 | (A) Number of studies per taxon and type of stressor. Publications that consider more than one stressor are represented independently for each stressor. (B) Diagram representing the number of studies within Anthozoa that applied more than one stressor.

and (2) the variation in response under different intensities of stress. Understanding those aspects would allow the identification of reliable and consistent gene biomarkers and improve the design of environmental stress experiments. We note that proteomic plasticity is also relevant to phenotypic plasticity and is tied to both transcriptional responses and post-transcriptional processes. While currently understudied, the increased accessibility of quantitative proteomics opens the possibility of investigating transcriptional and proteomic plasticity concurrently, enabling comparative assessment of the dynamics of the molecular response and how this correlates with phenotypic responses.

4.1 | Importance of Considering the Dynamics of the Transcriptional/Proteomic Response When Designing Experiments

Knowledge of the dynamics of gene and protein expression under environmental stress is necessary to inform plasticity and resilience and to design robust laboratory experiments that use gene and protein expression as tools to assess stress. As plasticity allows an organism to acclimate to different environments, analysing expression during acclimation and recovery after stress can help inform the dynamics of transcriptional and proteomic responses. Nevertheless, most

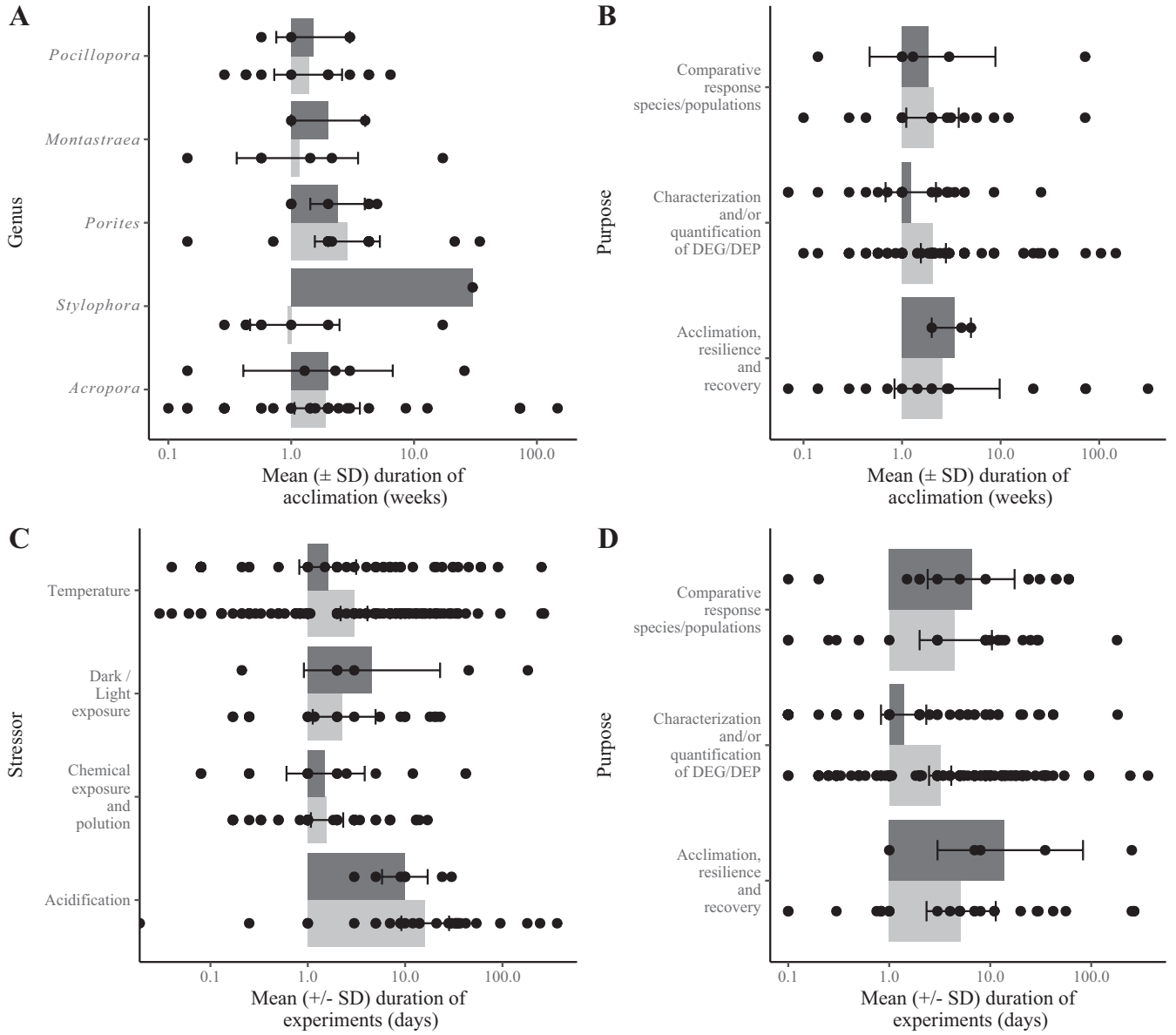

FIGURE 4 | Duration (mean ± SD) of exposure to stressors and acclimation. Dark grey bars represent studies that investigated protein expression; light grey bars represent studies that investigated gene expression; and dots represent individual studies. Publications that considered more than one stressor and/or performed both gene and protein expression analysis are represented independently. Note that the *x*-axis is presented as a log scale. (A) Duration of acclimation according to the most studied Anthozoa genera (92 articles). (B) Duration of acclimation according to the purpose of the study (152 articles). (C) Duration of exposure to stressors considering the four most common stressors studied (233 articles). (D) Duration of exposure to the four most common stressors considering the purpose of the study (209 articles).

studies on the responses of cnidarians to environmental stress did not sample during acclimation and recovery periods, and the duration of acclimation varied greatly among experiments (Figure 4). After being collected and/or handled, some species can return to baseline gene expression levels within 1 week (e.g., Bay and Palumbi 2015; Thomas et al. 2019), so relatively short acclimation periods are adequate. Other species, however, maintain differential expression for months after stress events (e.g., Pinzón et al. 2015; Thomas and Palumbi 2017; Thomas et al. 2019). In the last case, expression patterns observed during or after a laboratory stress experiment could be compromised, as the controls would not reflect the natural baseline gene expression of the species/population. While these studies have focused on gene expression, similar considerations would apply to protein expression studies (e.g., Barshis et al. 2018). If the acclimation period required for the organism studied is not known, we suggest sampling before the animal is collected/handled to establish its natural baseline and during acclimation/recovery to reveal expression plasticity of the population/species. This is important because stress experiments should only commence after the baseline of molecular expression has been re-attained (Figure 6).

The dynamics of gene/protein expression responses to a stressor may vary across species and populations (Figure 6). For that reason, even when an adequate acclimation period is used, different results may be obtained depending on the sampling points chosen in the experimental design. For example, in Figure 6, by

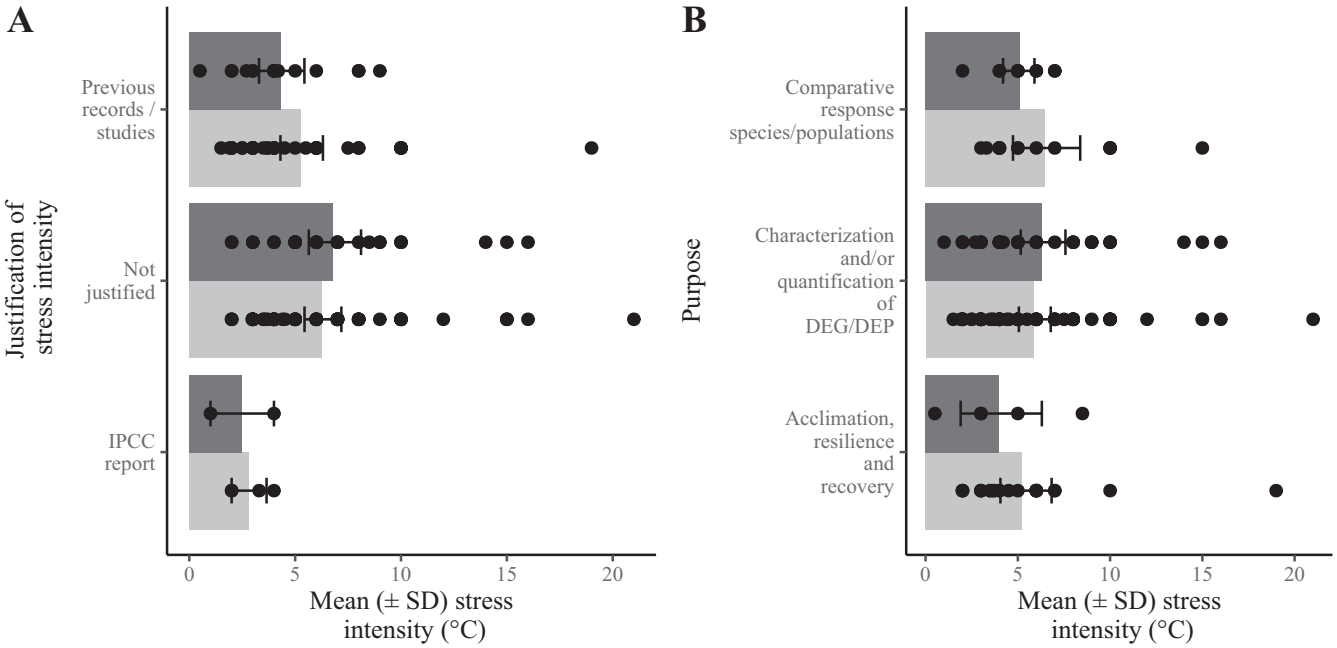

FIGURE 5 | Intensity of temperature stress in laboratory experiments according to the justification given by the authors on the methods for the stress intensity (A) and the purpose of the study (B) (153 articles included).

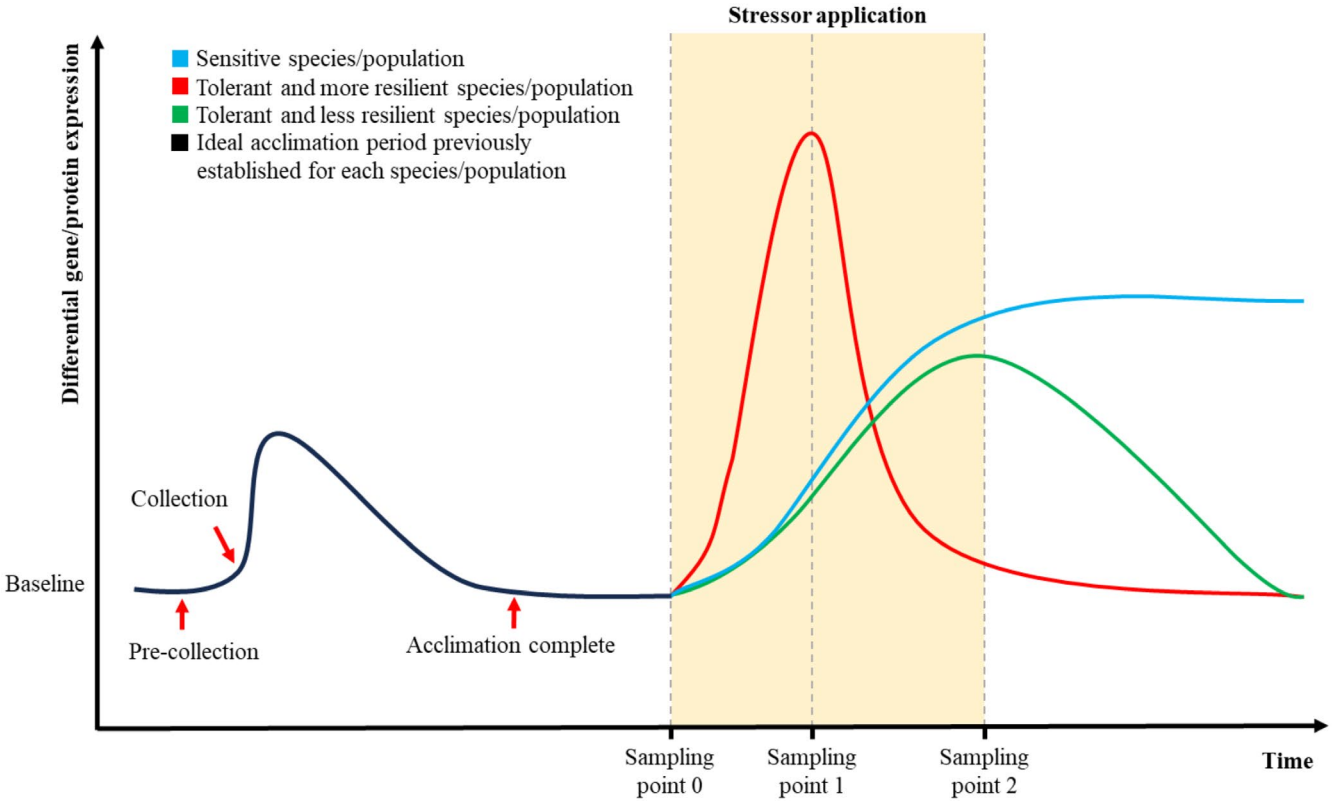

FIGURE 6 | Hypothetical differential gene/protein expression profiles of three species/populations during exposure to the same stressor. All species have the same baseline of gene/protein expression, and the acclimation period is idealised in the scheme.

sampling only before stress is initiated (sampling point 0) and at sampling point 1, the 'tolerant and more resilient' organism would be considered sensitive to the stressor in comparison to the other two. In contrast, if we sampled only before stress is introduced and at the end of the exposure (sampling points 0 and 2) the 'tolerant and less resilient' organism would be considered as sensitive as the 'sensitive' organism. The need for a careful sampling regime has been pointed out before (e.g., Rivera et al. 2021), and it is important, when logistically and financially possible, to increase the number of samples throughout the experiment to

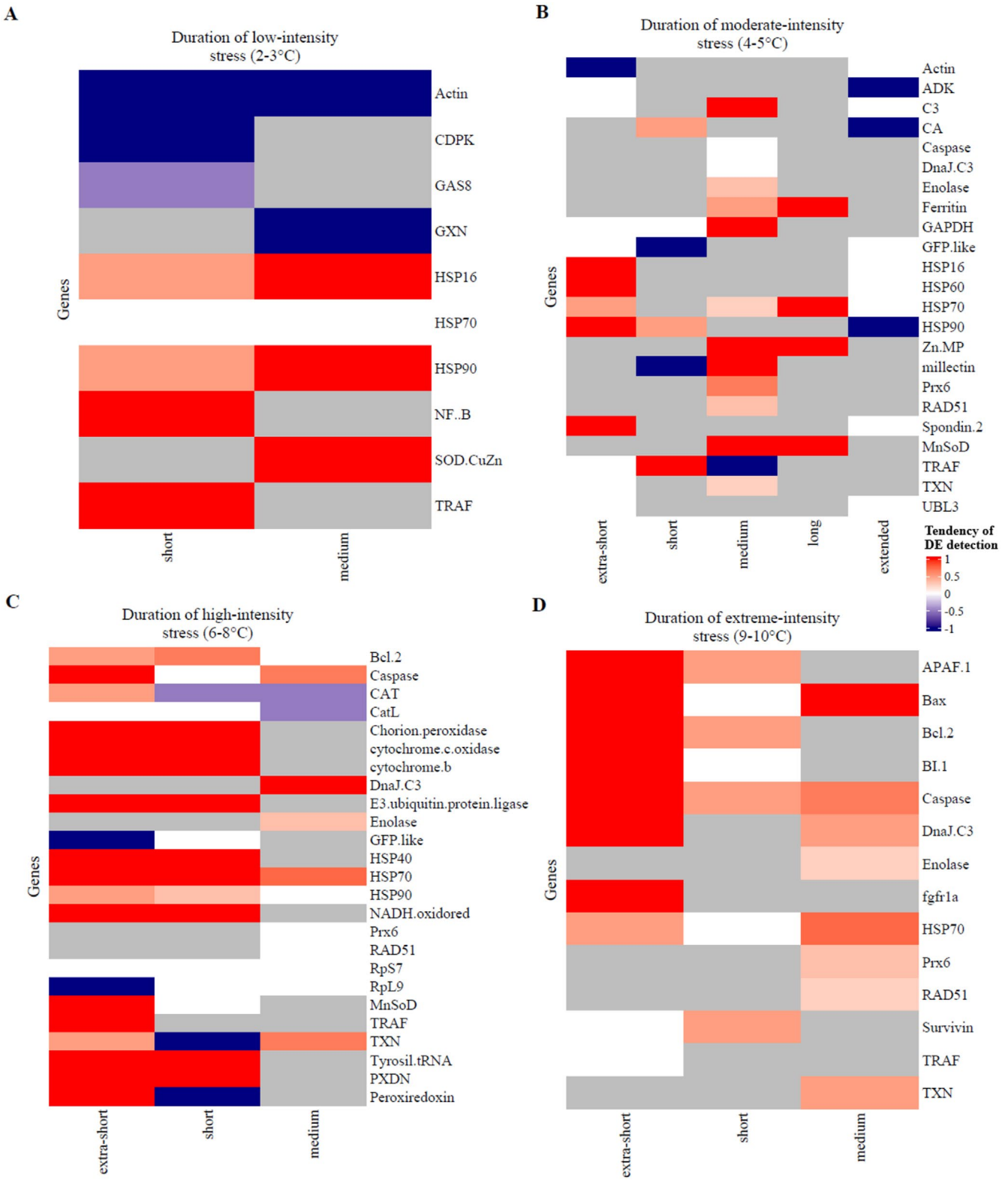

FIGURE 7 | Consistency of DE of genes across studies according to stress intensity and duration (see Section 2). Blue = tendency for downregulation. Red = tendency for upregulation. White = tendency for no significant change. Grey spaces represent the absence of data. Extra-short duration = equal or less than 1 day. Short duration = 2–7 days. Medium duration = 8–14 days. Long duration = 15–30 days. Extended duration = more than 30 days. Publications that performed more than one experiment are represented independently for each experimental condition.

determine the dynamics of expression profiles and make accurate inferences. Nevertheless, this procedure (sampling not only at the beginning and end, but also during the stress exposure) was only implemented by 39% of environmental stress experiments on cnidarians. If increasing the number of samples is not possible, we suggest performing pilot studies to reveal rates of molecular response to avoid erroneous conclusions and misinterpretation of the responses of species/populations to environmental stressors.

4.2 | Gene Biomarkers of Heat Stress in Cnidaria

Biomarkers are intended to be objective indicators of a response to environmental conditions (Parkinson et al. 2020), so they should have consistent responses under the same stressor and be representative of the stress response transcriptional profile (Figure 6). In our analysis of heat stress in the five most studied genera, only 14 out of 56 genes responded consistently to heat stress across studies (Table 1). Seven of those were tested in at least two different genera and may represent general cnidarian biomarkers associated with temperature stress; for that reason, we propose the acronym cBATS for this panel of genes. These are the immune response and/or oxidative stress genes: copper-zinc superoxide dismutase (CuZn-SOD), c-type lectin, fibroblast growth factor receptor 1 (FGFR1), matrix metalloproteinase (MMP), zinc metalloprotease (Zn-MP), nuclear factor kappa B (NF-κB) and solute carrier family 26 (SLC26). As cBATS must be reliable representations of stress response in cnidarians, these genes should be tested under different conditions of temperature stress to ensure they have a consistent and predictable response. FGFR1 was consistently upregulated in both *Acropora cervicornis* (Lamarck, 1816) and *Pocillopora acuta* Lamarck, 1816 after a 1-h extreme intensity (9°C) stress exposure, but its expression was not tested under other (more environmentally relevant) stress conditions in those genera. CuZn-SOD was upregulated in two experiments with low intensity (3°C) stress with different durations of exposure: 28 days with *Acropora hyacinthus* (Dana, 1846) and 12 days with *Montastraea cavernosa* (Linnaeus, 1767). To validate those two genes as cBATS, it is vital to test their differential expression under other environmental stress conditions.

The other five cBATS candidates showed a consistent differential expression response in at least two genera and under different heat stress situations. C-type lectin was downregulated in *A. cervicornis* after a 1-h extreme intensity (9°C) stress exposure, and in *Pocillopora damicornis* (Linnaeus, 1758) after a 15-day moderate (4°C) stress exposure. MMP was upregulated in *Montastraea faveolata* (Ellis & Solander, 1786) after a 10-day low intensity (3°C) stress exposure, and in *P. acuta* after a 16-h extreme intensity (9°C) stress exposure. Zn-MP was upregulated in *Acropora millepora* (Ehrenberg, 1834) under 9-days and 15-days of moderate intensity (5°C) stress exposure, and in *P. acuta* after a 4-day low intensity (2°C) stress exposure. NF-κB was upregulated in *A. millepora* after a 5-day moderate intensity (5°C) stress exposure, in *Acropora palmata* (Lamarck, 1816) after a 2-day low intensity (3°C) stress exposure, and in *P. acuta* after a 16-h extreme intensity (9°C) stress exposure and a 4-day low intensity (2°C) stress exposure. SLC26 was downregulated in *Porites astreoides* Lamarck, 1816 after a 42-day moderate intensity (4°C) stress exposure, and in *Stylophora pistillata* (Esper, 1792) after a 7-day high intensity (7°C) stress exposure. Although those genes had a consistent response across studies, they were all investigated in only two experimental situations, except for NF-κB, which was tested in four different experiments. More studies targeting those genes are necessary to confirm their differential expression consistency under heat stress. If confirmed, their use as biomarkers will facilitate cross-experimental comparisons on a large scale, possibly reducing the requirement for transcriptome-wide analyses, and provide a simple means to evaluate plasticity within transcriptomic responses.

Although there was some inconsistency in the response of genes that encode for heat shock proteins, they should still be further investigated as promising cBATS. Heat shock proteins are widely known as molecular chaperones that protect proteins from misfolding under stress conditions (Hu et al. 2022), and it is therefore not surprising that they were significantly upregulated in multiple studies during exposure to warm temperatures in the field and in laboratory experiments (Table 2). The few cases of downregulation or no significant change in gene expression of HSPs could be explained by post-transcriptional and translational regulation, as those are also key mechanisms for HSP expression (Hu et al. 2022). DeSalvo et al. (2010), for example, observed upregulation of HSP70 and HSP90 in *Acropora palmata* on the first day of 2.98°C intensity stress exposure, but no differential expression on the second day. Regardless of the importance of post-transcriptional processes, upregulation of HSPs can be used as a strong indicator of heat stress response in cnidarians. Additionally, as the expression of HSPs is not stressor specific (e.g., Schroth et al. 2005; Downs and Downs 2007), they can also be used to identify general stress in those animals. Indeed, as oxidative stress and immune response genes have been associated with general stress responses (Louis et al. 2017), differential expression of cBATS genes may be universally indicative of a broad range of stressors, not exclusively heat stress. For that reason, cBATS should also be investigated as universal environmental stress biomarkers for cnidarians.

Other genes that were relatively well-studied in cnidarians were upregulated, downregulated, and/or had no significant change in differential expression depending on the intensity and/or duration of heat stress exposure and on the species studied (Table 2). Carbonic anhydrase (CA), for example, was upregulated in an experiment with *Acropora* corals, but downregulated in *Montastraea*, *Porites* and *Stylophora*. Catalase (CAT), an oxidative stress gene, was upregulated in *A. millepora* corals collected in the field after heat stress but exhibited no significant change after a 9-day laboratory experiment with the same species. Genes with a highly dynamic expression profile might not be suitable as biomarkers of stress, as they can lead to a misinterpretation of the actual stress condition of the animal. For that reason, to identify reliable stress biomarkers for cnidarians, it is imperative that we first understand the plasticity and resilience of gene expression under environmental change. Establishing consistent biomarkers can improve management and conservation approaches and reduce costs, as it would optimise sequencing efforts focusing on validated target genes and proteins.

4.3 | Influence of Heat Intensity on Gene Expression of Cnidarians

Patterns of gene expression can vary under different intensities of environmental stress exposure (Table 2). Peroxiredoxin-6 (Prx-6), for example, was upregulated in *Acropora eurystoma* (Klunzinger, 1879) and *Porites* sp. corals exposed to a 13-day moderate (4°C) heat stress, but had no significant change in gene expression under high (8°C) and extreme (10°C) stress intensity exposure for the same duration (Maor-Landaw and Levy 2016). This highlights the need to perform environmentally relevant experiments (Hughes et al. 2017), with realistic stress intensities or, when possible, include multiple treatments with different

intensities to help identify the threshold of gene expression plasticity for the species/population. Although heat stress was widely investigated in cnidarians, stress intensities applied in laboratory-based experiments varied greatly (Figure 5), and only 31% of studies used more than one intensity.

High-intensity stress in cnidarians was previously associated with a generally consistent stress response (type A, Dixon et al. 2020), with upregulation of genes involved in cell death, response to reactive oxygen species, NF-κB signalling, immune response, protein folding and protein degradation (Ruiz-Jones and Palumbi 2017; Dixon et al. 2020). In contrast, low-intensity stress has been linked to more variable responses (type B, Dixon et al. 2020), sometimes opposite to the type A response. In our analysis of gene biomarkers, we noted a tendency for upregulation of all genes tested in experiments under extreme-intensity stress (9°C–10°C), but inconsistencies in some genes tested under low, moderate and high-intensity stress, depending on the duration of the stress exposure (Table 2; Figure 7). Millectin, for example, was up-or down-regulated in *Acropora* corals under moderate-intensity stress depending on the duration of the exposure (Barshis et al. 2013; Van De Water et al. 2015). As cBATS should have a consistent and reliable response under various environmental stress conditions, they must also have a consistent differential regulation under both type A and B responses to stress.

4.4 | Combining Gene and Protein Expression Analysis With Other Response Variables

Changes in gene transcription often precede metabolic, physiological and behavioural changes in an organism under environmental stress. The lag between molecular response rates and the response rates of other commonly used variables to assess stress can limit/impede the use of both molecular and other variables in the same experiment. For example, an experiment designed to investigate changes in gene expression under thermal stress might consider sampling during the first 2 days of stress exposure to address plasticity and identify the first line of response. This period might not be sufficient to observe changes in reproduction, for example (e.g., strobilation and budding in moon jellyfish, that can occur more than a week after the temperature is reduced, Fuchs et al. 2014). If the study intends to compare variables with different rates of response to environmental stress, it might be necessary to increase the number of sampling points to capture those rates, or tailor the sampling of different variables to times appropriate for each variable.

4.5 | General Trends of the Studies Assessed and Recommendations for Future Studies

The high conservation value of corals and the impacts of climate change on these animals justifies the disproportionate focus on the responses of Anthozoa to heat stress. Nevertheless, research efforts have focused on just four genera of anthozoans (*Acropora*, *Seriatopora*, *Montastraea* and *Porites*). Although several factors influence the selection of an organism to study (e.g., abundance in the environment, permits, knowledge of husbandry), species often respond differently to the same stressor, and the response of broadly studied organisms might not be the same as others (Pitt et al. 2018; Bertile et al. 2023). When possible, less studied taxa should be considered for experiments to increase knowledge on molecular pathways related to the environmental stress response of cnidarians and determine the extent of the similarities in responses across different species and groups.

Key aspects of the experimental designs, including intensity of stress, duration of exposure, sampling frequency and acclimation period, varied greatly between studies assessed in this review. This inconsistency compromises the comparison of results obtained by different studies and shows that there is still a need to establish adequate procedures to study the transcriptomic and proteomic responses to stress in cnidarians. Although it would be unreasonable to advocate that all researchers conform to similar experiments, designing experiments that mimic timelines of actual events (e.g., specific heatwaves) may lead to more similar and, therefore, comparable designs. Validating reliable biomarker genes and proteins (cBATS) would also facilitate comparison of results, optimise sequencing efforts and reduce costs. Experimental designs should be appropriately described, with clear justifications for intensities and durations of exposure to environmental stressors. We encourage reviewers to ensure adequate descriptions and justifications by the authors on their experimental design, and journals to require appropriate data annotation in submitted data sets, as data availability is critical to facilitate meta-analysis that reveals broader trends across studies.

5 | Conclusions

There is a great inconsistency between how experiments that study transcriptomic and proteomic responses of cnidarians to environmental stress have been conducted. The variability in experimental designs, even between articles with the same focus of study, limits the comparison of results. As gene and protein expression can vary under different stress profiles (considering variations in intensity and duration of exposure to the stressor, for example), expression plasticity must be accounted for when establishing intensity of stress, sampling frequency and acclimation period in an experiment. As plasticity allows an organism to acclimate to different environments and recover from stress, we suggest designing experiments that include these two periods, with multiple sampling points through acclimation and/or recovery. When budgets are limited, pilot studies would help detect the dynamics of expression profiles before planning and designing stress experiments. Experiments should also be environmentally relevant and appropriately described, with realistic stress intensities (and multiple treatments with different intensities, if possible), so the results could be used to inform future management and conservation approaches.

Author Contributions

All authors conceived the idea and edited the article. C.G.M. conducted the literature review, performed the analyses and wrote the article.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw data are available in the Supporting Information section of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.